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PRINCIPAL INVESTIGATOR: Peter S. Nelson

CONTRACTING ORGANIZATION: Fred Hutchinson Cancer Research Center
Seattle, WA 98109

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Peter S. Nelson

E-Mail: pnelson@fhcrc.org

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14. ABSTRACT

The identification of recurrent, protein-altering genetic alterations is frequently the means by which a given gene is initially implicated in tumor biology. Our specific aims are as follows: (1) Carry out the genome-wide identification of nonsynonymous mutations in a limited number of prostate metastases using second-generation technologies for targeted capture and sequencing; (2) Evaluate the mutational histories of individual mutations within the progression of the cancer in which it was observed, and to assess the prevalence of candidate cancer genes observed here in prostate cancer. (3) Perform integrative analyses of somatic mutation with gene expression and copy number change data collected on the same samples. During this funding period we have: (i) completed the identification and quality assessment of the prostate cancer tissues/samples that will be used for the duration of the project; (ii) completed DNA isolation for 204 samples/cases of prostate cancer that will be used for the project; (iii) transferred the first group of DNAs to the Shendure lab for Exome analysis; (iv) participated in interpreting the Exome sequence data for the first 23 samples. These studies have produced the exciting and novel result identifying a 'hyper-mutated' prostate cancer phenotype; (v) completed copy number analyses for 70 prostate cancers; (vi) identified, and confirmed, recurrent point mutations in several genes and genetic pathways; (vii) initiated network-based integrated molecular analyses of advanced prostate cancers that includes copy-number variation and transcript profiles.

15. SUBJECT TERMS
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Introduction

The identification of recurrent, protein-altering genetic alterations is frequently the means by which a given gene is initially implicated in tumor biology. However, we currently lack a comprehensive picture of the protein-altering mutations that are biologically relevant or potentially specific to prostate cancer. The research supported by this award aims to use a new generation of technologies for DNA sequencing to comprehensively scan the genomes of a series of prostate cancers for small mutations that disrupt protein-coding sequences. Our specific aims are as follows: (1) To carry out the genome-wide identification of nonsynonymous mutations in a limited number of prostate metastases using second-generation technologies for targeted capture and sequencing; (2) To evaluate the mutational histories of individual mutations within the progression of the cancer in which it was observed, and to assess the prevalence of candidate cancer genes observed here in prostate cancer. (3) To perform integrative analyses of somatic mutation with gene expression and copy number change data collected on the same samples.

Body

This is a "synergy" project between the laboratories of Dr. Jay Shendure in the Department of Genome Sciences at the University of Washington (UW) and Dr. Peter Nelson in the Division of Human Biology at the Fred Hutchinson Cancer Research Center (FHCRC). Because these are separate awards to the two investigators, this progress report is specific to tasks from the statement of work (SOW) assigned to the Nelson Lab only (or to progress within the Nelson Lab for joint tasks). As per the instructions, progress is reported in association with each of the relevant tasks listed in the SOW.

Aim 1: Perform a comprehensive screen for protein coding alterations in prostate metastases.

Task 1. Select tumors to be subjected to exome sequencing (Months 1-2) [FHCRC]

In Year 1 of this project, we obtained 204 prostate cancer specimens. These included 30 primary prostate cancers, 24 prostate cancer xenografts primarily representing advanced prostate cancer, and 150 metastatic prostate cancers. The metastatic prostate cancers represent multiple metastasis (5 each) from each of 30 patients. Each tumor sample was embedded in OCT, sectioned, and evaluated for percentage of tumor by hematoxylin and eosin staining and pathological review. Task Complete.

Task 2. Tumor tissue processing and quality control (Months 2-3) [FHCRC]

From each tumor specimen, sections were cut on a cryostat and RNA and DNA was extracted (Qiagen micro RNA and DNA purification columns/reagents). The quantity of RNA and DNA was determined by NanoDrop methods. The quality of RNA and DNA was determined by Agilent Bioanalyzer assays. In total, 5 samples did not pass q/c assessments for a total success rate of 96%. DNA for exome capture and sequencing studies was transferred to the Shendure Lab. Task Complete.

Task 3. DNA isolation and shotgun library construction (Months 4-10) [UW]

Performed in Shendure lab—see companion Progress Report.

Task 4. Array-based enrichment of coding sequences (Months 7-13) [UW]

Performed in Shendure lab—see companion Progress Report.

Task 5. Massively parallel sequencing of tumor and control exomes (Months 10-16) [UW]

Performed in Shendure lab—see companion Progress Report.

Task 6. Read mapping, variant calling, and mutation annotation (Months 11-17) [UW]

Performed in Shendure lab—see companion Progress Report.

Aim 2: Evaluate mutational histories and prevalence screen of candidate cancer genes.

Task 7. Design & testing of MIP-based capture assay. (Month 18-20) [FHCRC]

Work on this Task has just commenced (ahead of schedule) and is based on the identification of novel non-synonymous nucleotide variants (nov-SNVs) identified from the first 24 exome sequences. These nov-SNVs are detailed in the Progress Report from the Shendure lab, and include: p53 mutations, SPOP mutations, and MSH6 mutations. Note, although originally assigned as a task to the Nelson lab, the MIP-based capture assay will be performed in the Shendure lab.

Task 8. Selection and q/c of validation tissues (Month 8-16) [FHCRC]

We have identified a cohort of 300 primary prostate cancers with attendant formalin fixed paraffin-embedded tissues from the pathology archives at the University of Washington. Each of these cases was selected based on outcomes data of relapse versus non-relapse following radical prostatectomy. For 200 of these cases, we have completed a pathology review to identify cancer and benign prostate regions, and obtained a 'punch' of these corresponding tissues that will be used for DNA preparations. The evaluation of the remaining 100 cases is ongoing. For an additional 30 prostate cancer cases, comprised primarily of metastasis, we have verified the histology, and completed the isolation of DNA (see Task 9).

Task 9. Preparation of DNA for validation tissues (Month 9-18) [FHCRC]

For 30 prostate cancer cases (in addition to those used in Aim 1 above) we have completed DNA extraction. Q/C of A260/280 ratios was performed and all samples passed this analysis. The preparations of DNA from the first 200 primary prostate cancers with outcomes will begin next month and will be followed by the remaining 100 cases.

Task 10. Application to evaluate mutation histories (Months 20-24) [UW]

This task will be performed primarily in the Shendure lab—see companion Progress Report. However, to perform initial verification/validation studies, we have evaluated the mutation calls for several genes discovered initially by the Exome capture and NextGen sequencing (see Shendure Progress Report). For example, we have confirmed the exome calls for mutations in the *speckle-type POZ protein* (SPOP) gene, involved in mediating SRC-3/AIB1 activity—and consequently the activity of the androgen receptor---and determined that this gene is recurrently mutated in prostate cancers (**Figure 1**).

Sample ID	Description	hg18	NT change	Protein Change	Validated by Norm
98-328J	retroperitoneal LN met	Chr17:45051431	A->C	131 Silent	Yes
05-116F	lung met	Chr17:45051425	A->C	F133V	Yes
03-027P2	liver met	Chr17:45051643	A->C	F102D	Yes
03-082H1	liver met	Chr17:45051642	A->C	F102C	Yes
00-010L	retroperitoneal LN met	Chr17:45051642	A->C	F102C	Yes

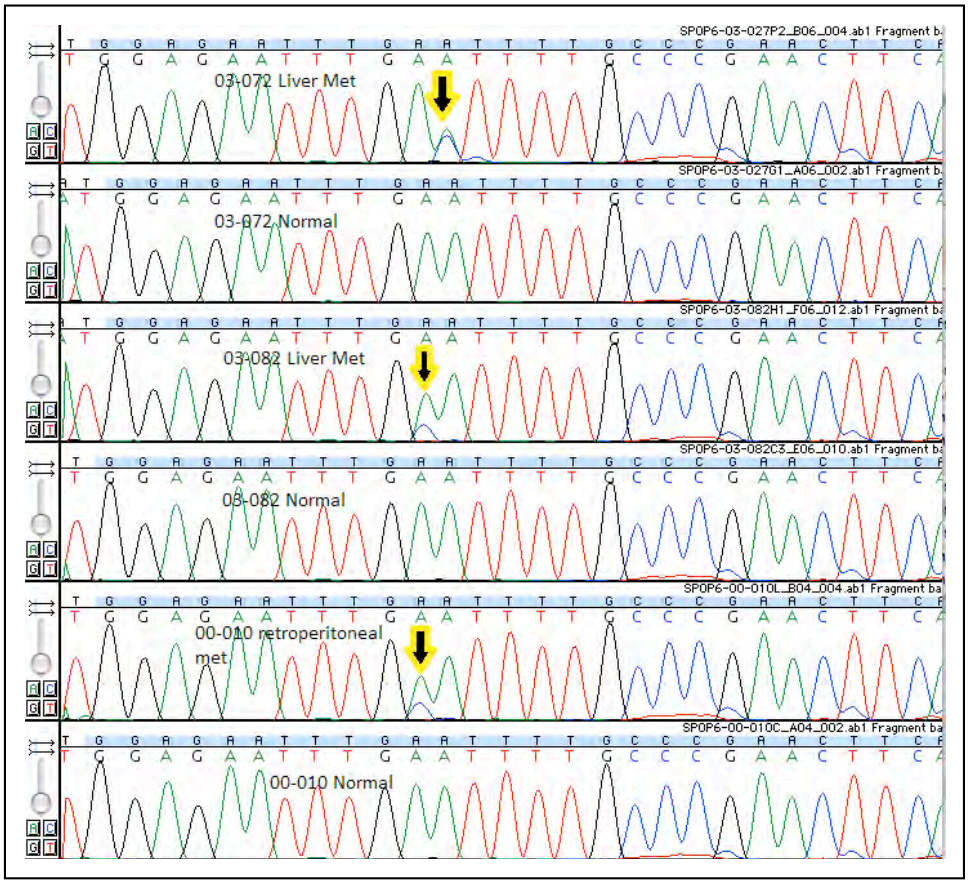

Figure 1. Determination of recurrent mutations in the SPOP coding region. Mutations in the binding pocket of the SPOP protein were identified initially in 2/24 prostate cancer samples. These mutations were confirmed in 4 of 30 additional cases with one additional (presumed) silent mutation (Top panel). Actual Sanger sequence traces confirm exome-based sequence mutations (Bottom panel).

Task 11. Application to prevalence screen of candidate cancer genes (Months 18-30) [UW]

To be performed in Shendure lab.

Task 12. Read mapping, variant calling, and mutation annotation (Months 21-31) [UW]

To be performed in Shendure lab.

Task 13. Verification/confirmation of sequence variants (Months 20-26) [UW]

To be performed in Shendure lab.

Aim 3: Integrate analyses of molecular alterations in metastatic and primary prostate cancer.

Task 14. Development of algorithms for integrative analysis (Months 1-12) [FHCRC]

In collaboration with Dr. Pei Wang, and the recently established SAGE bioinformatics group located at FHCRC (http://sagebase.org/), we have been developing approaches to homogenize disparate datasets the provide complementary information regarding signaling pathways operating in prostate cancers. These take advantage of network-based analyses using coherent datasets (e.g. data from the same sample) involving transcript profiles, copy number variation, and nucleotide sequence (e.g. mutation) analyses. We are currently assessing the output of these algorithms for defining key activity nodes that can be modulated for therapeutic targeting. Figure 2 depicts a network map based on transcript profiles and copy number alterations in prostate cancer that center on the androgen receptor (AR) and a co-regulator NCOA2. In progress is the integration of mutation data into this network.

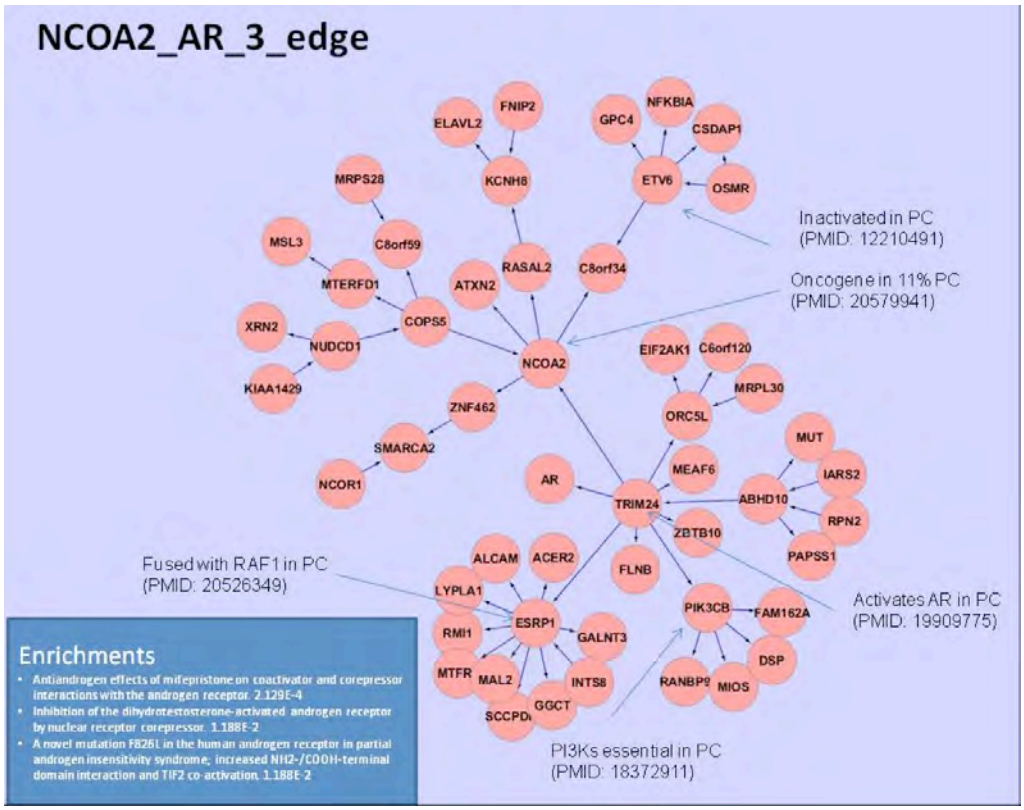

Figure 2. Network structure of prostate cancer molecular alterations using coherent data from primary and metastatic prostate cancers. Note the node centering on NCOA2 that is a putative target for therapeutics.

Task 15. Analysis of mutational patterns in metastatic prostate cancer (Months 11-17) [UW]

To be performed in Shendure lab.

Task 16. Analysis of mutational patterns in primary prostate cancer (Months 21-31) [UW]

To be performed in Shendure lab.

Task 17. Analysis of copy number alterations in prostate cancer (Months 6-31) [FHCRC]

We have now completed the analysis of copy number variation (CNV) across 70 prostate cancers (using DNA extracted from samples in Aim 1, a subset of which have also been used for mutation analyses by exome sequencing). We have identified several genomic regions with recurrent alterations including regions in 8p, 8q, PTEN, RB, NCOA2, and AR. **Figure 3** shows 3 genomic regions with high frequencies of CNVs across 24 prostate cancers.

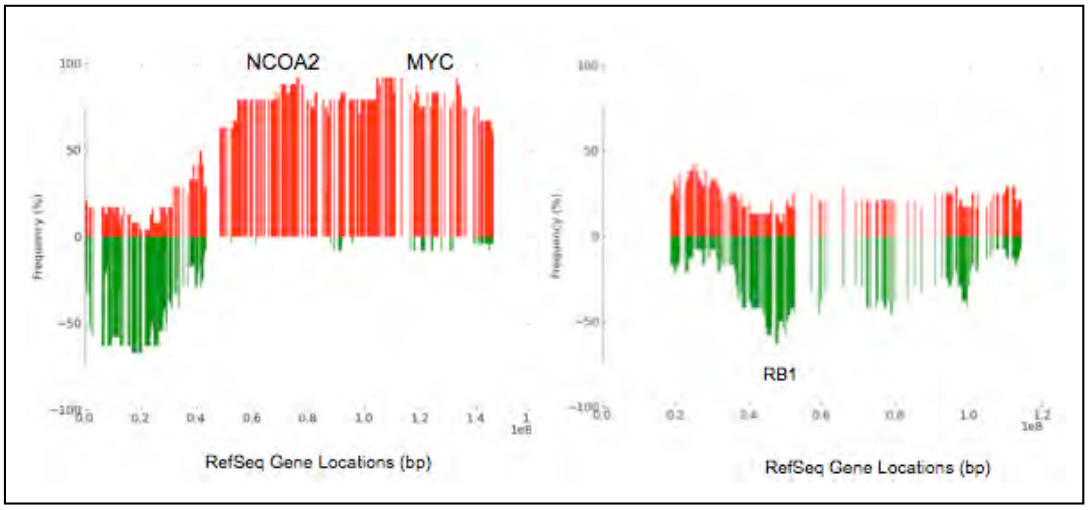

Figure 3. Array CGH analyses of 24 prostate cancers. Regions shown are those with high frequency (up to 50%) of genomic alterations. These involve relevant prostate cancer-associated genes: NCOA2, MYC, and RB1.

Task 18. Analysis of consequences of genome alterations (protein/mRNA) (Months 18-28) [FHCRC]

To be started in Month 18. The identification of samples and RNA preparations as substrate for these studies has been initiated.

Task 19. Integrative pathway analysis of DNA alterations. (Months 8-34) [FHCRC]

We have initiated the integration of coherent prostate cancer data as described and depicted under Task 14 above. In process is the integration of mutation/polymorphism data into these network maps. The refinement of these integration maps will continue throughout the duration of the proposal.

Task 20. Integrative analysis for RNA expression and DNA alterations (Months 18-34) [FHCRC]

To be started in Month 18. The identification of samples and RNA preparations as substrate for these studies has been initiated.

Task 21. Annotating and posting project data to website (Months 18-36) [UW & FHCRC]

To be performed in conjunction with Shendure lab.

Task 22. Completing project reports and manuscripts (Months 11-36) [UW & FHCRC]

To be performed in conjunction with Shendure lab.

Key Research Accomplishments

- We have completed the identification and quality assessment of the prostate cancer tissues/samples that will be used for the duration of the project.
- We have completed DNA isolation for 204 samples/cases of prostate cancer that will be used for the project.
- We have transferred the first group of DNAs to the Shendure lab for Exome analysis.
- We have participated in interpreting the Exome sequence data for the first 23 samples. These studies have produced the exciting and novel result identifying a 'hyper-mutated' prostate cancer phenotype.
- In collaboration with the Shendure lab we have identified, and confirmed, recurrent point mutations in several genes and genetic pathways. These include recurrent mutations in p53 and in the ubiquitin ligase component SPOP.
- We have completed array CGH analyses of copy number variation across 70 prostate cancers.
- We have initiated network-based integrated molecular analyses of advanced prostate cancers that includes copy-number variation and transcript profiles.

Reportable Outcomes

- In June 2011, we submitted a manuscript entitled "Exome Sequencing Identifies a Spectrum of Mutation Frequencies in Advanced and Lethal Prostate Cancers" to the Proceedings of the National Academy of Sciences (PNAS), describing our progress on this project to date. The reviews were generally positive, and the manuscript has been revised and resubmitted to address the comments of the reviewers. The manuscript is not included here because the paper has not yet been accepted.
- Preliminary findings for this project were reported in an invited talk (given by Dr. Shendure) at the 17th Annual Scientific Retreat of the Prostate Cancer Foundation, (Washington DC).

Conclusion

In summary, by sequencing the exomes of 23 tumors representing a spectrum of aggressive advanced prostate cancers, we identified a large number of previously unrecognized gene coding variants with the potential to influence tumor behavior. However, our results also indicate that with notable exceptions, very few genes are mutated in a substantial fraction of tumors. Furthermore, while the overall mutation frequencies approximate those found in other cancers of epithelial origin, we also identified a distinct subset of tumors that exhibit a hypermutated genome. It will be important to determine the mechanism(s) responsible for the enhanced point mutation rates in these malignancies, particularly if further studies demonstrate enhanced resistance to cancer therapeutics.